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A new species of *Nicolea* (Annelida, Terebellidae) from Qingdao, China

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Abstract

Terebellidae is a large group of tubicolous annelids commonly found in benthic marine environments worldwide, from shallow to deep waters. Within this family, the genus *Nicolea*, which comprises 30 accepted species, has been widely reported around the world, yet only a single species has been recorded in Chinese waters. Here we describe a new species *Nicolea qingdaoensis* **sp. nov.**, collected from Qingdao, China. The new species is distinguished from congeners by a combination of morphological characters: presence of eyespots; two pairs of branchiae on segment 2–3, with the first pair much larger than the second; 17 thoracic notopodia; ventral shields on segment 2 to 17; completely intercalated double rows of uncini starting from segment 11; genital papillae on segments 6 and 7 of equal length but sexually dimorphic, small spherical in females and digitiform in males. Phylogenetic analyses based on the concatenated dataset of *cox1*, *16S*, *18S*, *28S* and *H3* genes revealed that *N. qingdaoensis* **sp. nov.**, was sister to the clade including *N. venustula* (Montagu, 1819) from Mediterranean Sea, and *N. gracilibranchis* (Grube, 1878) from Hawaii and an unidentified *Nicolea* from Redondo Beach, California, USA. Additionally, we provided the complete mitochondrial genome of the new species.

Key Words

Chinese coasts, marine benthos, mitochondrial genome, polychaete, Terebellinae

Introduction

Species in Terebellidae are tubicolous worms distributed widely in marine benthic ecosystems, ranging from shallow water to deep-sea sediments (Hutchings et al. 2020; Lavesque et al. 2021; Quintanar-Retama et al. 2022). The genus *Nicolea* was erected by Malmgren (1866), with *Nicolea zostericola* (Grube, 1860) described from the Koster Islands, Sweden designated as the type species. According to the most recent comprehensive diagnosis of all terebellid genera by Hutchings et al. (2020), species of *Nicolea* are characterized by the following characters: absence of lateral lobes, two pairs of arborescent branchiae with short main stalks, notopodia from segment 4 continue for a variable number of segments from 18 to 43 with narrowly-winged notochaetae, neuropodia from segment 5 to pygidium, uncini avicular initially in single rows, double rows from segment 11 until end of notopodia then in single rows again, nephridial papillae on segment 3 and genital papillae on segments 6 and 7.

Species of *Nicolea* have a global distribution, with records from the Cape of Good Hope (South Africa), Malay Archipelago, East India, Australia, New Zealand, Sweden, UK, Mediterranean, and China (Table 1). The genus currently comprises 30 accepted species (Hutchings et al. 2021; Read and Fauchald 2025; Table 1), 12 of which

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have their type localities in the Western Pacific (Table 1). These species include six described from Southeast Asia: *N. gracilibranchis* (Grube, 1878) and *N. claparedii* (Grube, 1878), from the Philippines, *N. angustiscutis* Caullery, 1944, *N. longibranchia* Caullery, 1944 and *N. willeyi* Caullery, 1944, from the Malay Archipelago and *N. koehleri* Caullery, 1944, from Borneo; three species from coastal waters of Australia: *N. amnis* Hutchings & Murray, 1984, *N. murrayae* Nogueira, Hutchings & Carrerette, 2015 and *N. vaili* Nogueira, Hutchings & Carrerette, 2015; two species from New Zealand: *N. maxima* Augener, 1923 and *N. armilla* Hutchings & Smith, 1997; and one, *N. sinensis* Fauvel, 1932, from the coastal waters of Yantai, China.

The phylogenetic position of *Nicolea* in Terebellidae remains unresolved. Historically, *Nicolea* was placed in the subfamily Amphitritinae (Day 1967; Holthe 1986; McHugh 1995), which was later synonymized with Terebellinae (Nogueira et al. 2013). Morphological studies have produced conflicting phylogenies. McHugh (1995) recovered *Nicolea* as sister to *Ramex* Hartman, 1944, a genus characterized by possessing only a single pair of branchiae, while Garraffoni and Lana (2008) placed it in a clade containing *Hadrachaeta* Hutchings, 1977, *Laphania* Malmgren, 1866 and *Ramex*. A subsequent morphological analysis suggested that *Nicolea* was the earliest branching clade within Terebellinae (Nogueira et al. 2013). This phylogenetic uncertainty is compounded by a lack of molecular data. Currently, only four *cox1* sequences from identified *Nicolea* species have been deposited in GenBank, which are inadequate for reliable species delimitation in this genus. Furthermore, the molecular phylogeny is challenged by broader unresolved taxonomic issues within Terebellidae (Hutchings et al. 2021). Herein, we describe a new species *Nicolea qingdaoensis* sp. nov., collected from Qingdao, China, and provide its complete mitochondrial genome (mtgenome) along with sequences of commonly used nuclear markers (i.e., *18S*, *28S*, *H3*). The phylogenetic position of the new species was investigated using both *cox1* and a multi-locus concatenated dataset.

Materials and methods

Sample collection and morphological examination

A total of 19 specimens of *N. qingdaoensis* sp. nov., were collected from the intertidal rocky shore near the "Deep Blue Light" (new Zhanqiao Pier) (36.05519°N, 120.34604°E), Hongshiya (36.08363°N, 120.09666°E) and Haizhifeng (36.06299°N, 120.38952°E) in Qingdao, China, from 2024 to 2025 (Suppl. material: table S1). Photos of living specimens were taken by Zeiss SteREO Discovery V20 with a digital camera (Carl Zeiss Microscopy GmbH). The whole specimen of QD240920SP2_01 and the tail of QD_HZF_20250920, QD_Terebellidae_sp2_002_250924 and QD_Terebellidae_sp2_003_250924 were used for DNA extraction and next-generation sequencing (NGS). The others were fixed and preserved in 75% ethanol for morphological studies and deposited at the Marine Biological Museum of the Chinese Academy of Sciences (MBMCAS), situated within the Institute of Oceanology, Chinese Academy of Sciences (IOCAS) with the catalog numbers of MBM287946–MBM287950. The whole specimens, including detailed morphology of the notochaetae and uncini, were observed with an Olympus SZX16 stereomicroscope and photographed using Canon EOS 5D Mark IV. The holotype was stained with saturated methyl green solution. For scanning electron microscopy (SEM), the tissue with notochaetae and uncini was dehydrated in a series of graded ethanol, and replaced with hexamethyl disilazane (HMDS) solutions. After drying in a fume hood, the tissue was attached to the sample stand by carbon tape, then scanned by JSM-7100F electron microscope. The specimens of *N. sinensis* under MBM190135 (one male, collection information is missing) and MBM199353 (one female and five males, collected in Daheilan, Qingdao, Shandong, on Feb. 28, 1964) were also checked. The terminology used for species morphology follows Nogueira et al. (2010) and Hutchings et al. (2020).

Molecular data and analysis

Specimens or tissues preserved in 99% ethanol were sent to Novogene (Beijing, China) for DNA extraction and library construction. Next-generation sequencing was performed on the DNBSEQ-T7 platform (paired-end sequencing with read length 150 bp). The quality of raw reads was assessed using FastQC v0.12.1 (Brown et al. 2017) integrated in the FQC Dashboard, under the default settings. Low-quality reads were removed by Trimmomatic-0.39 (Bolger et al. 2014). *De novo* assembly was performed using SPAdes-3.15.4 with default settings. Target nuclear loci (*18S* rRNA, *28S* rRNA, and *H3*) were retrieved from contigs generated by SPAdes v3.15.4 with BLAST V2.15.0 (Camacho et al. 2009). It was performed using published *18S*, *28S*, and *H3* sequences of Terebellidae as a reference database, separately, with an e-value threshold of < 1e-5. Where necessary, overlapping short sequences were merged into the longer consensus sequence using CAP3 (Huang and Madan, 1999). Sequence integrity was further verified by: (1) multiple sequence alignments with MAFFT (Katoh et al. 2017), and (2) preliminary phylogenetic analysis. Mitochondrial genome was assembled using NOVOPlasty 4.3.1 (Dierckxsens et al. 2017) with key parameters as follows: the mtgenome of *Terebella lapidaria* (OX424571.1) served as the seed sequence; a K-mer length of 33; an insert size of 300 bp (with automatic fine-tuning enabled); and a target size range of 12,000–22,000 bp. The generated mtgenome was annotated on the MITOS2 web server using code "Invertebrate (5)" and reference "RefSeq89 Metazoa" (Bernt et al. 2013). The positions of start/stop codons of 13 PCGs were manually checked by aligning them with the published terebellid mtgenomes.

Table 1. Morphological information on the species of *Nicolea*.

	Body length(mm)/ wide(mm)/ segment	Notopodia counts (pairs)	number of segments with ventral shields (start-end)	Description of ventral shields	Notopodia	Eyespots	Branchiae on segment 2 and 3	Branchiae appearance
***Nicolea amnis* Hutchings & Murray, 1984**	12/1.5	16	14, 2–15.	NA	NA	P	1 = 2	Each with only 2–3 dichotomous branches.
***Nicolea angustiscutis* Caullery, 1944**	30/4/70	17	13, 2 to 14–28	Gradually narrowing.	NA	A	1 = 2	1st long (reaching segment 5–6); 2nd sessile, massive and arborescent.
***Nicolea armilla* Hutchings & Smith, 1997**	85/6/75	15	NA	NA	Rectangular, all similar in size except for 1st	A	1 > 2	Robust, annulated stalks with pale speckles; left branchia on segment 2 regenerating.
***Nicolea ceciliae* Santos, Nogueira, Fukuda & Christoffersen, 2010**	24/2.1	17	14–15, 2–15/16	Shields narrow posteriorly from segment 10.	Notopodia with brown line at tip	P	1 > 2	Conspicuous annulated stems; 1st longer and inserted more dorsally.
***Nicolea chilensis* (Schmarda, 1861)**	45/-/-	17–22	14, 2–15	NA	NA	A	1 > 2	Reddish-gray; the upper branchiae bearing 6 branches, the lower branchiae with 5 branches
***Nicolea claparedii* (Grube, 1878)**	45/3.5/87	23–25	14, 2–15	Anterior shields broad and rectangular; posterior transversely oval.	NA	NA	NA	1st branches 5 or 6, widely spaced, length diminishing rapidly toward the apex, 2nd branch more divided.
***Nicolea dasycomus* (Grube, 1866)**	37/8/46	21–25	16, 2–17	NA	NA	NA	1 > 2	NA
***Nicolea gracilibranchis* (Grube, 1878)**	50/3.5–7/70	17–21	16, 2–17	Anterior shields subequal; posterior shields narrow.	NA	NA	1 > 2	Highly compound, a very slender stem and branches,1st branchiae with 9 distant branches.
***Nicolea gracilicauda* (Kinberg, 1866)**	NA	NA	NA	NA	NA	NA	3	Branchiae shorter than body width.
***Nicolea incerta* Caullery, 1944**	-/1.5/-	17	14, 2–15	NA	Notopodia from small to large and then back to small	NA	1 > 2	NA
***Nicolea koehleri* Caullery, 1944**	-/1.5/-	17	NA	NA	NA	A	1 > 2	Antler-shaped with widely spaced main branches from dichotomous base.
***Nicolea latens* Chamberlin, 1919**	-/4/-	17	NA	Anterior shields clearly separated; posterior separations weak.	NA	P	1 > 2	Reddish brown color.

	Body length(mm)/ wide(mm)/ segment	Notopodia counts (pairs)	number of segments with ventral shields (start-end)	Description of ventral shields	Notopodia	Eyespots	Branchiae on segment 2 and 3	Branchiae appearance
***Nicolea lazowasemi* Nogueira, 2008**	12/2.04/42	15	14, 2–15	Shields rectangular on segments 2–15, larger anteriorly.	First two pairs of notopodia shorter than following pairs.	A	1 > 2	With dichotomous branchings, ending in short and middle-sized filaments
***Nicolea lobulata* Hartmann-Schröder, 1965**	75/4/69	NA	NA	Shields transition from rectangular to trapezoidal.	NA	A	1 = 2	Branchiae exhibiting dichotomous branching patterns, with elongated and robust primary stem.
***Nicolea longibranchia* Caullery, 1944**	20/2/51	16–17	NA	Ventral shields widest at segment 7.	NA	A	1 = 2	NA
***Nicolea longicornis* (Sars, 1829)**	25.4/6.4/-	NA	NA	NA	NA	P	NA	Two short dichotomous branched gills
***Nicolea macrobranchia* (Schmarda, 1861)**	90/-/-	NA	NA	NA	Elongate chaetal bundles extend to segment 26.	NA	1 > 2 > 3	Intensely red; 1st with 6 branches, 2nd with 5 primary branches.
***Nicolea maxima* Augener, 1923**	300/16/106	16–18	NA	NA	NA	A	1 > 2	1st short stalk, 5 branches; 2nd sessile, 4 branches.
***Nicolea modesta* Verrill, 1900**	-/-/56	17	NA	NA	NA	NA	1 > 2	Small, sparsely branched with short stalks and 2 lateral lobes.
***Nicolea murrayae* Nogueira Hutchings & Carrerette, 2015**	5/1.2	14	12, 2–13	Smooth, trapezoidal shields on 2–13, narrower posteriorly.	Notopodia of 1st about same size as following pairs and originate slightly dorsally to them.	P	1 > 2	Branchial filaments with few dichotomous branches.
***Nicolea profundi* (Chamberlin, 1919)**	48/4.2/	17	11, 2–12	NA	NA	A	NA	NA
***Nicolea qingdaoensis* sp. nov.**	22/1.5/61	17	16, 2–17	Shields to segment 17, then replaced by a mid-ventral groove.	Gradually increase from segment 4 to 20	P	1 > 2	Branchial filaments with more dichotomous branches.
***Nicolea schmardai* Holthe, 1986**	60/-/118	21	NA	NA	NA	NA	NA	1st with 4 branches, 2nd with 3 branches.
***Nicolea simplex* Verrill, 1873**	19/2.6/-	17	14, 2–15	Broad anterior shields narrowing posteriorly, becoming longer than wide.	Notopodia short, conical; first 2 pairs with shorter chaetae than following pairs, dorsally aligned to them.	A	1 > 2	Short common stem, dichotomous, terminating in short filaments.

	Body length(mm)/ wide(mm)/ segment	Notopodia counts (pairs)	number of segments with ventral shields (start-end)	Description of ventral shields	Notopodia	Eyespots	Branchiae on segment 2 and 3	Branchiae appearance
***Nicolea sinensis* Fauvel, 1932**	40/4/70	17	13–17, 2–14/18	NA	Notopodia rectangular, with notochaetae directing to dorsal side.	P	1 > 2	Slender dense terminal branches; stalks annulated, poorly developed distally.
***Nicolea uspiana* (Nogueira, 2003)**	21/2/54	17	19, 2–20	Shields broader anteriorly, narrowing posteriorly; ruffled anteriorly, uniform from segment 4.	NA	P	1 > 2	Dichotomous.
***Nicolea vaili* Nogueira Hutchings & Carrerette, 2015**	7.7/0.7/41	17	14, 2–15	Shields transition from rectangular to square, and finally to long and narrow.	Notopodia originating progressively more laterally until segment 8, then longitudinally aligned.	P	1 > 2	Branchial filaments with few dichotomous branches.
***Nicolea venustula* (Montagu, 1819)**	31/1/51	17, rarely 18 or 19	13, 2–14	NA	NA	P	1 > 2	Dichotomous branched with long stems on segments 1 and 3.
***Nicolea viridis* (Schmarda, 1861)**	60/-/118	21	NA	NA	NA	NA	NA	Light green; first with 6, second with 5 branches.
***Nicolea willeyi* Caullery, 1944**	-/1.5/-	17	19, 2–20	NA	Notopodia uniform in width, and the terminal somites not significantly longer	P	1 > 2	Dichotomous branching with tapered ends.
***Nicolea zostericola* (Grube, 1860)**	20/0.2/50	15	15, 2–16	NA	NA	P	1 > 2	Very short stalks, few dichotomies, and very few terminal branches.

	Double rows uncini start from	Notochaetae	Uncini	Upper lip	Lower lip	Nephridial papillae and genital papillae	Type location	Synonymized species
***Nicolea amnis* Hutchings & Murray, 1984**	10	Notochaetae narrow-winged broad-bladed capillaries, within a bundle; chaetae of 2 lengths.	NA	NA	NA	NA	NSW, Australia	*Nicolea minima* Hartmann-Schröder, 1984

	Double rows uncini start from	Notochaetae	Uncini	Upper lip	Lower lip	Nephridial papillae and genital papillae	Type location	Synonymized species
***Nicolea angustiscutis* Caullery, 1944**	NA	NA	Wide base, 2 large teeth plus a small apical denticle. Frontally, 4 equal teeth above large lower one.	NA	NA	NA	Waigeo Island, Malay Archipelago	NA
***Nicolea armilla* Hutchings & Smith, 1997**	10	NA	MF:2–3:2–3	Upper lip withdrawn.	Lower lip rectangular, rugose and partially hidden.	Nephridial papillae on segment 3; genital papillae segment 6–7.	New Zealand, Matheson Bay, near Leigh, North Island	NA
***Nicolea ceciliae* Santos, Nogueira, Fukuda & Christoffersen, 2010**	10	Narrowly-winged notochaetae on both tiers, those on posterior tier with wing starting from mid-length of chaetae.	Short handle, triangular heel, mid-length button, crest with 2 rows of secondary teeth.	Upper lip longer than wide.	Lower lip swollen, cushion-like.	Nephridial papillae on segment 3, genital papillae on segments 6 and 7.	Rio Grande do Norte, Brazil	NA
***Nicolea chilensis* (Schmarda, 1861)**	9	Notochaetae with smooth tips.	Arranged in alternating double rows on thoracic segments (excluding anteriormost).	NA	NA	Nephridial papillae on segment 3, genital papillae on segments 6 and 7.	South Africa	*Phyzelia* (*Terebella*) *chilensis* Schmarda, 1861
***Nicolea claparedii* (Grube, 1878)**	10	NA	Uncini aviculares	NA	NA	NA	Philippines	*Terebella claparedii* Grube, 1878
***Nicolea dasycomus* (Grube, 1866)**	NA	NA	NA	NA	NA	NA	Saint-Paul, MN, USA	*Terebella dasycomus* Grube, 1866
***Nicolea gracilibranchis* (Grube, 1878)**	11	NA	NA	NA	NA	NA	Philippines	*Terebella gracilibranchis* Grube, 1878
***Nicolea gracilicauda* (Kinberg, 1866)**	NA	Limbate (flattened and blade-like), narrow, acute, elongated.	Short, bifid (bidentate) tip in lateral view, strongly curved.	NA	NA	NA	Tahiti near Papeete	*Terebella gracilicauda* Kinberg, 1866
***Nicolea incerta* Caullery, 1944**	NA	NA	4	NA	NA	NA	East India	NA
***Nicolea koehleri* Caullery, 1944**	NA	NA	3–4	NA	Lower lip well developed.	NA	Borneo	NA

	Double rows uncini start from	Notochaetae	Uncini	Upper lip	Lower lip	Nephridial papillae and genital papillae	Type location	Synonymized species
***Nicolea latens* Chamberlin, 1919**	NA	Curved distally	Primary tooth series commonly with 7–9 teeth.	NA	NA	NA	Gulf of California, USA	NA
***Nicolea lazowasemi* Nogueira, 2008**	11	Notochaetae on both sides with narrow limbation, broader at 1 margin	Short, triangular heel, well-developed dorsal button.	Upper lip short, hood-like, higher than broad.	Lower lip swollen.	Nephridial papillae on segment 3, genital papillae on segments 6 and 7.	Long Island Sound, Double Beach, Branford, New Haven County, Connecticut, USA	NA
***Nicolea lobulata* Hartmann-Schröder, 1965**	10	NA	Thoracic uncini have 4 denticles and larger; abdominal ones have 2.	NA	NA	NA	Chile	NA
***Nicolea longibranchia* Caullery, 1944**	9	NA	NA	Slightly raised dorsally.	Expanded	NA	Malay Archipelago	NA
***Nicolea longicornis* (Sars, 1829)**	NA	NA	NA	NA	NA	NA	Bergen, Norway	NA
***Nicolea macrobranchia* (Schmarda, 1861)**	NA	Dorsal notopodial chaetae exhibit sigmoidal curvature.	NA	Upper lip indistinctly bilobed with a recurved margin.	Lower lip has 2 smaller lateral processes with a shallow transverse groove.	NA	Cape of Good Hope	*Terebella macrobranchia* Schmarda, 1861
***Nicolea maxima* Augener, 1923**	10	NA	NA	NA	NA	NA	Akaroa Harbour, New Zealand	NA
***Nicolea modesta* Verrill, 1900**	10	Notochaetae slender and smooth, 3–4 longer and 2–4 shorter.	NA	NA	NA	NA	Bailey Bay, Ketchikan, Alaska	NA

	Double rows uncini start from	Notochaetae	Uncini	Upper lip	Lower lip	Nephridial papillae and genital papillae	Type location	Synonymized species
***Nicolea murrayae* Nogueira Hutchings & Carrerette, 2015**	10	Notochaetae in both rows narrowly-winged distally.	Uncini throughout with ~3 rows of progressively shorter secondary teeth.	Peristomium forming lips, hood-like upper lip.	Hood-like upper lip, longer than wide; small, swollen lower lip, restricted to oral area.	Nephridial papillae on segment 3, genital papillae on segments 6 and 7, females with rounded papillae, males with elongate, digitiform papillae.	Lizard Island, Qld Australia	NA
***Nicolea profundi* (Chamberlin, 1919)**	12	NA	NA	NA	NA	NA	Aguja Point, Peru	NA
***Nicolea qingdaoensis* sp. nov.**	11	Notochaetae, broad-winged capillaries, with fine tips, tilted at an angle.	MF:3–4:2–3	Upper lip nearly oval, periphery transparent in live, boundaries not obvious after alcohol preservation.	Lower lip small and inconspicuous.	Genital papillae on segments 6 and 7.	Qingdao, China	NA
***Nicolea schmardai* Holthe, 1986**	NA	Weakly curved, with a winged margin.	Hook-shaped, short stalk, bearing 4 progressively smaller teeth.	NA	NA	NA	Ceylon	*Phyzelia viridis* Schmarda, 1861
***Nicolea simplex* Verrill, 1873**	11	Notochaetae on both sides with narrow limbation, broader at 1 margin.	Triangular heel, dorsal button, crest with single row (2 teeth).	Upper lip about as broad as high, nearly circular.	Lower lip restricted to oral area, button-like.	Nephridial papillae on segment 3, genital papillae on segments 6 and 7, elongate, digitiform, originating posteriorly to notopodia and vertically aligned to them, those on segment 7 longer.	New Haven to Vineyard Sound, northeastern, USA	NA
***Nicolea sinensis* Fauvel, 1932**	10 in Fauvel 1932, 11 in Nishi et al., 2014 and specimens checked in this study	Notochaetae dorsal capillary, narrow-margined, single-tipped.	MF:2–3:1–2	NA	NA	Segment 6–9, long cylindrical in male and glandular in female	Yantai, China	NA

	Double rows uncini start from	Notochaetae	Uncini	Upper lip	Lower lip	Nephridial papillae and genital papillae	Type location	Synonymized species
***Nicolea uspiana* (Nogueira, 2003)**	10	Almost smooth under highest magnification of light microscope (1600×), very minutely denticulate under SEM.	MF:2–4:4–5	Upper lip upwardly directed, distally rounded.	NA	Genital papillae on segments 6 and 7.	State of São Paulo: Ubatuba, Praia da Fazenda, Brazil	*Paraeupolymnia uspiana* Nogueira, 2003
***Nicolea vaili* Nogueira Hutchings & Carrerette, 2015**	10	Notochaetae in both rows narrowly-winged distally.	With ~3 rows of progressively shorter secondary teeth.	Hood-like upper lip as long as wide.	Small, swollen lower lip, restricted to oral area.	Nephridial papillae on segment 3, genital papillae on segments 6 and 7.	Lizard Island, Qld, Australia	NA
***Nicolea venustula* (Montagu, 1819)**	11	Dorsal chaetae smooth-margined capillary chaetae.	Uncini with 1 large and several smaller teeth.	Upper lip low, curving around and dorsally and laterally covering the mouth.	NA	Nephridial papillae on segment 3, genital papillae on segments 6 and 7, those of segments 6 and 7 of ripe males prolonged and tube-like.	Southern coast of Devon at Torcross, England	*Nicolea viridis* Webster & Benedict, 1884; *Nicolea arctica* Malmgren, 1866
***Nicolea viridis* (Schmarda, 1861)**	NA	NA	Short, hook-shaped, uniform width, 4 distally diminishing denticles.	NA	NA	NA	Massachusetts, USA	*Phyzelia viridis* Schmarda, 1861
***Nicolea willeyi* Caullery, 1944**	NA	NA	3 teeth: the basal denticle not very developed; the vertex several denticles distinguishable.	NA	NA	Nephridial papillae on segment 3, genital papillae on segments 4–6.	Malay Archipelago	NA
***Nicolea zostericola* (Grube, 1860)**	NA	NA	NA	Upper lip thick, curving around the mouth and covering it dorsally and laterally.	NA	Nephridial papillae on segment 3, genital papillae on segments 6 and 7; those of segment 6 and 7 of ripe males prolonged.	Koster Islands, Sweden	NA

A = absent. "NA" indicates that the literature is not documented.

Phylogenetic trees were reconstructed based on two datasets: the *cox1* gene dataset, and the concatenated dataset of *cox1*, *16S*, *18S*, *28S* and *H3* genes. The *cox1* dataset contains 107 sequences from 96 species (including 26 unidentified species) in 28 genera (Suppl. material: table S2). The concatenated gene dataset comprised a total of 152 sequences from 109 species in 31 genera (Suppl. material: table S2). Terebellidae sp. (PV263602.1) is not included because of its poor identification. Three species of *Pectinaria* (i.e., *P. lizhei* Zhang, Hutchings & Qiu, 2022, *P. torquata* Zhang & Qiu, 2017, and *P. dayaensis* Zhang, Hutchings & Qiu, 2022 were selected as outgroups (Suppl. material: table S2), considering they belong to Pectinariidae, a family closely related to Terebellidae within the suborder Terebelliformia.

Sequences of each gene were aligned using the MAFFT version 7 under default settings (Katoh et al. 2017). The best model is predicted under Akaike information criterion (AIC) for Maximum Likelihood (ML) analyses and Bayesian Information Criterion (BIC) for Bayesian Inference (BI) analysis using the model prediction module in PhyloSuite v1.2.3, and ModelFinder is used for the *cox1* gene dataset and PartitionFinder2 for the concatenated dataset. The GTR+R+F was used for ML analyses and HKY+I+G+F for BI analysis for the *cox1* dataset. For the concatenated dataset, aligned sequences of each gene were concatenated using PhyloSuite v1.2.3 (Zhang et al. 2020). Each gene in the concatenated dataset was treated as a single partition. The GTR+I+G was used for *cox1*, *16S* and *H3* and TRN+I+G for *18S* and *28S* for both ML and BI analysis for the concatenated dataset. ML analyses were conducted by the IQ-TREE (Minh et al. 2022) in PhyloSuite v1.2.3 with 1,000 Ultrafast Bootstrap (UFBoot) replicates with the SHaLRT test. The BI tree was conducted using MrBayes in PhyloSuite v1.2.3 (Zhang et al. 2020). The MCMC chains were run for 200,0000 generations, with a sampling frequency of 1000 replicates. The consensus tree was constructed with the first 25% of samples discarded as burn-in. The tree files were visualized using FigTree v1.4.4. The sequence identity and pairwise distance (p-distance) were calculated using Megalign 7.1.0.44 within DNAstar Lasergene 7.1 (DNASTAR Inc., WI, USA).

Data availability

The raw sequencing data generated from NGS (5.64 Gb of QD240920SP2_01, 5.03 Gb of QD_HZF_20250920, 4.71 Gb of QD_Terebellidae_sp2_002_250924, 4.29 Gb of QD_Terebellidae_sp2_003_250924) have been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA1377332. The GenBank accession numbers of sequences of mtgenome, *18S*, *28S* and *H3* are shown below: PX527395, PX621371–PX621373 for mitochondrial genome, PX575633, PX617814–PX617816 for *18S*, PX575634, PX617811–PX617813 for *28S*, PX619625–PX619628 for *H3*.

Taxonomy

Terebellidae Johnston, 1846

Nicolea Malmgren, 1866

Type species. *Nicolea zostericola* (Grube, 1860)

Nicolea qingdaoensis sp. nov.

https://zoobank.org/2E5F02DC-952C-48C0-A76E-DF1C090074E7

Figs 1–4

Materials examined. ***Holotype.*** • MBM287946, complete, female, rocky shore near the "Deep Blue Light" (new Zhanqiao Pier), Huangdao, Qingdao, China (36.05519°N, 120.34604°E), low tide zone (tide lowest at 56 cm), September 20, 2024.

Paratypes. • MBM287947, four specimens (two males and two females), same collection date and site as the holotype. • MBM287949, eight specimens (four males and four females), same collection site as the holotype, low tide zone (tide lowest at 89 cm), September 17, 2025. • MBM287948, four specimens (two males and two females), Hongshiya (36.08363°N, 120.09666°E), rocky shore at high tide zone (tide lowest at 94 cm), Qingdao, China, September 24, 2025. • MBM287950, one specimen, incomplete, female, Haizhifeng (36.06299°N, 120.38952°E), low tide zone (tide lowest at 92 cm), Qingdao, China, September 20, 2025.

Additional material. • QD240920SP2_01, complete, same location and collection date as the holotype, whole worm used for molecular analyses.

Description. Measurements. Holotype MBM287946 (QD_Tere_sp2_002_20240920) with 61 segments, 22 mm long, 1.5 mm wide. Paratypes: complete specimens with 55 to 67 segments, body length from 9 to 24 mm, body width between 1 and 2 mm (Suppl. material: table S1). Tube constructed of sand, small gravel and shell fragments, inner membrane thick and robust (Fig. 1A). Live worm salmon or yellow or reddish in color with white pigments (Fig. 1B, C, D), becoming paler after preservation in ethanol (Fig. 1E). Buccal tentacles long, grooved, uniformly light yellow.

Prostomium at base of dorsal surface of upper lip (Fig. 1F); basal part with rows of lateral eyespots (Fig. 1E). Peristomium forming lips, upper lip hood-like, higher than wide, reddish with yellow margins in living specimen; lower lip small, rectangular, rugose, restricted to oral area (Fig. 1F).

Segment 1 reduced dorsally, developed ventrally (Fig. 1F). Paired dorso-lateral arborescent branchiae on segments 2 and 3. The first pair double length of second pair, with a short basal stem inserted slightly more dorsally (Fig. 1B, C, G). Anterior segments slightly inflated dorsally, and lateral lobes absent (Fig. 1H, I). Rounded nephridial papillae on segment 3, at base of branchiae (Fig. 1I). Genital papillae on segments 6 and 7, of equal size, spherical in females (Fig. 1J) and digitiform in

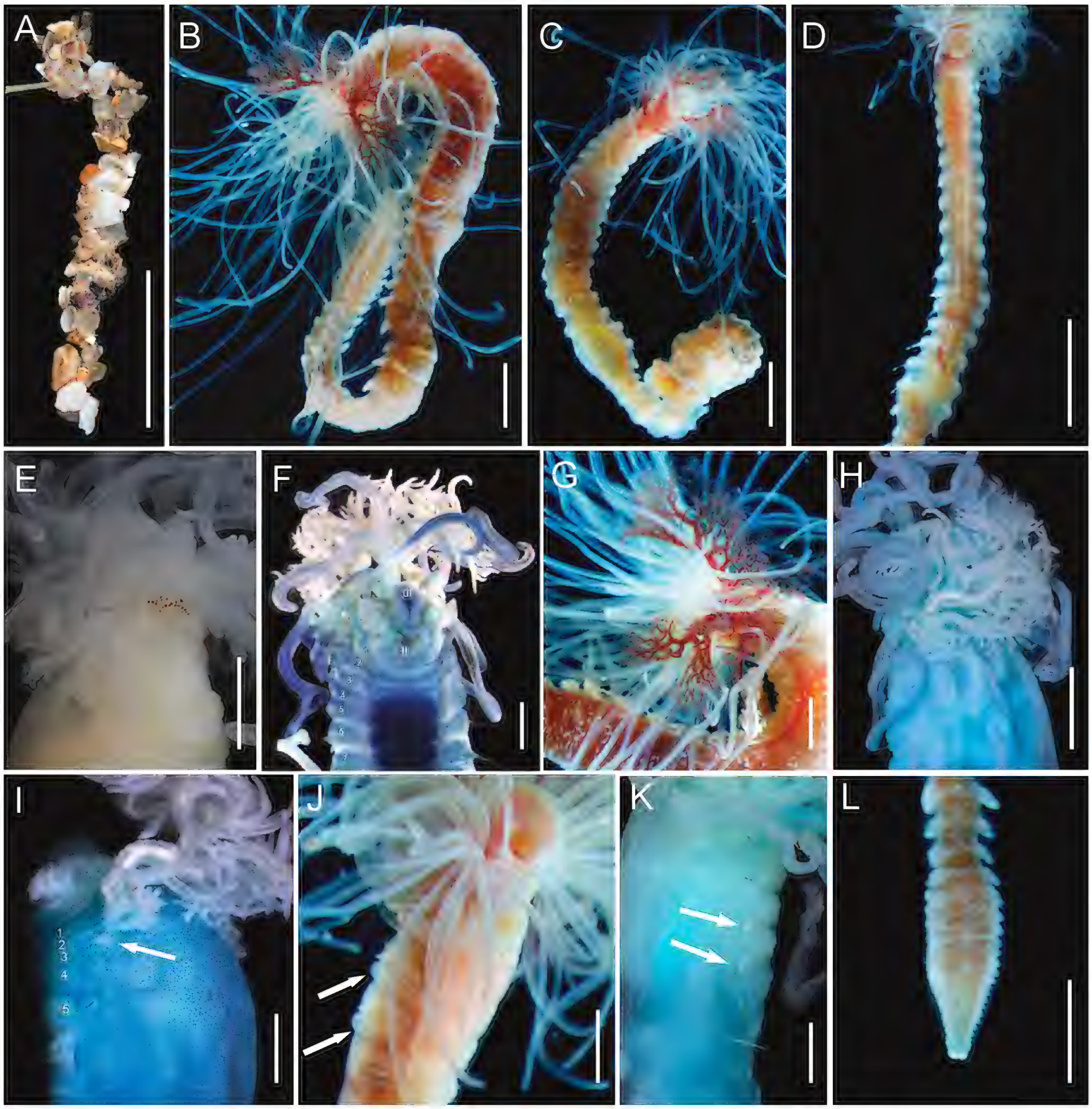

Figure 1. *Nicolea qingdaoensis* sp. nov. **A.** Tube morphology; **B, C.** Living whole worm, dorsal view; **D.** Living whole worm, ventral view; **E.** Anterior, lateral view, showing the eyespots; **F.** Anterior, ventral view; **G.** Anterior of living worm, dorsal view, showing the branchiae; **H.** Anterior, lateral view; **I.** Anterior, showing the nephridial papillae; **J.** Anterior, showing the genital papillae, female; **K.** Anterior, showing the genital papillae, male; **L.** Living worm, abdomen. **A, E.** QD_Tere_sp2_04 (MBM287947); **B–D, G, J, L.** Holotype MBM287946; **F, H, I, K.** QD_Tere_sp2_05 (MBM287947). **B, C, D, G, J, L.** Living specimens; **E.** Preserved in alcohol; **F, H, I.** After methyl green staining. Scale bars: 10 mm (**A**); 500 µm (**B, C, D, L**); 0.5 mm (**E**); 2 mm (**F, K**); 250 µm (**G, I, J**); 1 mm (**H**).

males (Fig. 1K). Ventral shields on segments 2–17, all smooth, rectangular, about the same width on segments 2–7, progressively narrower and longer from segment 8 onwards, replaced by mid-ventral groove from segment 18 and extending until pygidium (Fig. 1D, L).

Seventeen pairs of short and conical notopodia from segment 4 to 20 (Fig. 1D). Neuropodia from segment 5, with sessile ridges until segment 20, slightly raised pinnules or elongated in abdomen (Fig. 1D, K, L), becoming shorter and smaller posteriorly (Fig. 1L).

Notochaetae narrowly-winged, with fine tips, tilted at an angle (Fig. 2A, B). Neurochaetae in single rows on segments 5–10 (Fig. 2C–G). Uncini short-handled, with short triangular heel, distally rounded prow, downwardly directed process present, dorsal button situated at mid-length between base of main fang and tip of prow (Fig. 2C, D); main fang large, sharply pointed, with 2 rows of shorter secondary teeth basally (MF:3–4:2–3) (Fig. 2E, F). Uncini arranged in completely intercalated double rows from segments 11 to 20 (Fig. 2H), in single rows

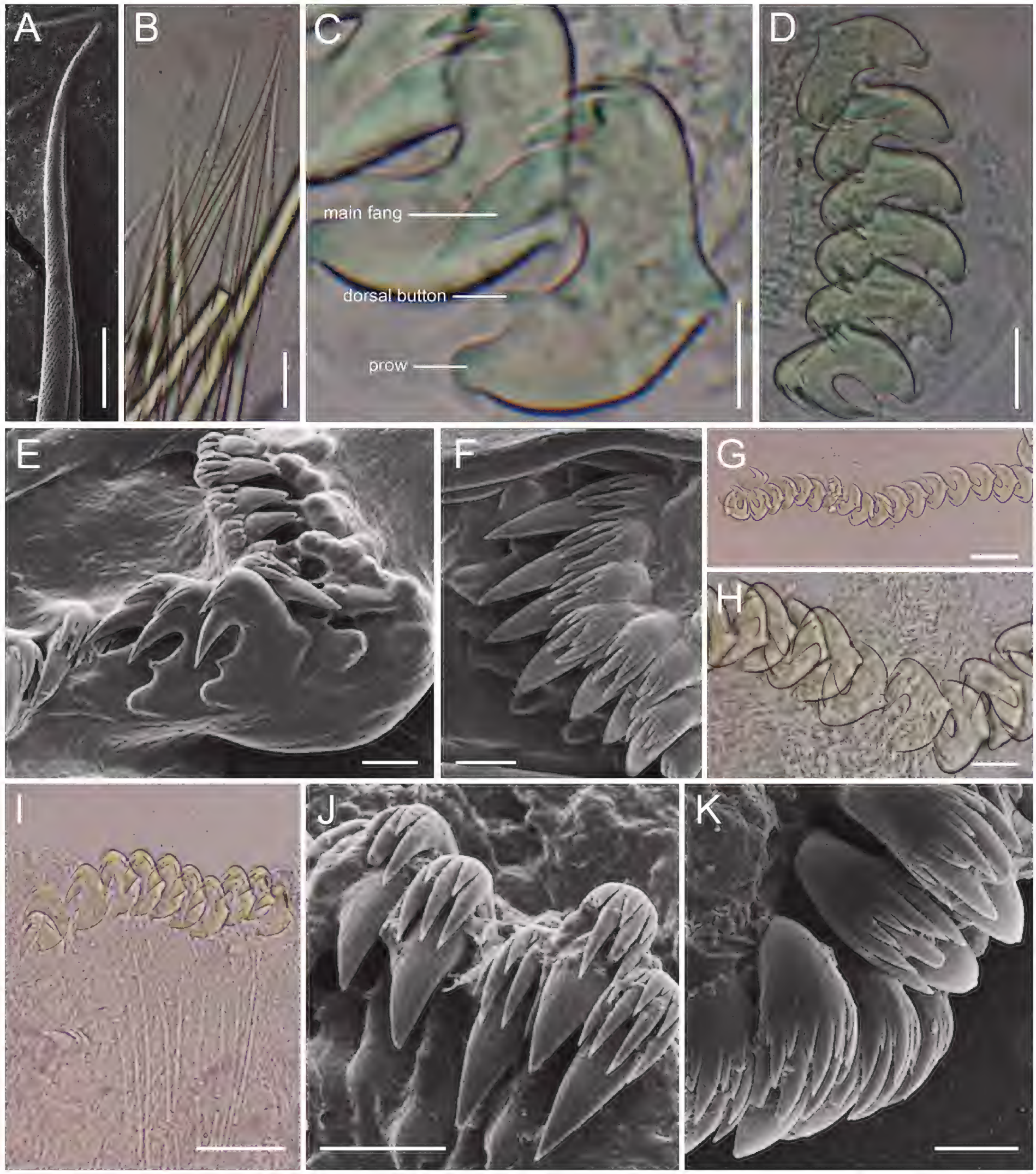

Figure 2. Uncini and notochaetae of *Nicolea qingdaoensis* sp. nov. Holotype MBM287946. **A.** Notochaetae, segment 8; **B.** Notochaetae, segment 8; **C.** Uncini, segment 7; **D.** Uncini, segment 7; **E.** Uncini, segment 8; **F.** Uncini, segment 8; **G.** Uncini, segment 10; **H.** uncini, segment 18; **I.** Uncini, segment 42; **J.** Uncini, posterior segment; **K.** Notochaetae, posterior segment. Scale bars: 10 µm (**A, C, J, K**); 40 µm (**B, G, I**); 20 µm (**D, H**); 30 µm (**E**); 5 µm (**F**).

from 21 onwards (Fig. 2I). Uncini from posterior segments similar to those of thorax (Fig. 2J, K). Pygidium smooth (Fig. 1L).

Variation. Specimens yellowish-white or light green after being preserved in ethanol (Fig. 3A–H; Suppl. material: fig. S1). Specimen QD_HZF_20250920, male, bears small sphere-shaped papillae on branchiae and abdominal neuropodia (Fig. 3A, E; Suppl. material: fig. S1.1H, L). Number of rows of eyespots varies from 1 to 5 (Suppl. material: table S1). Holotype with fewer and lighter-colored eyespots compared to other specimens (Suppl. material: table S1).

Etymology. The species name '*qingdaoensis*' refers to the type locality Qingdao.

Type locality. New Zhanqiao Pier (Deep Blue Light), Huangdao, Qingdao, Shandong, China.

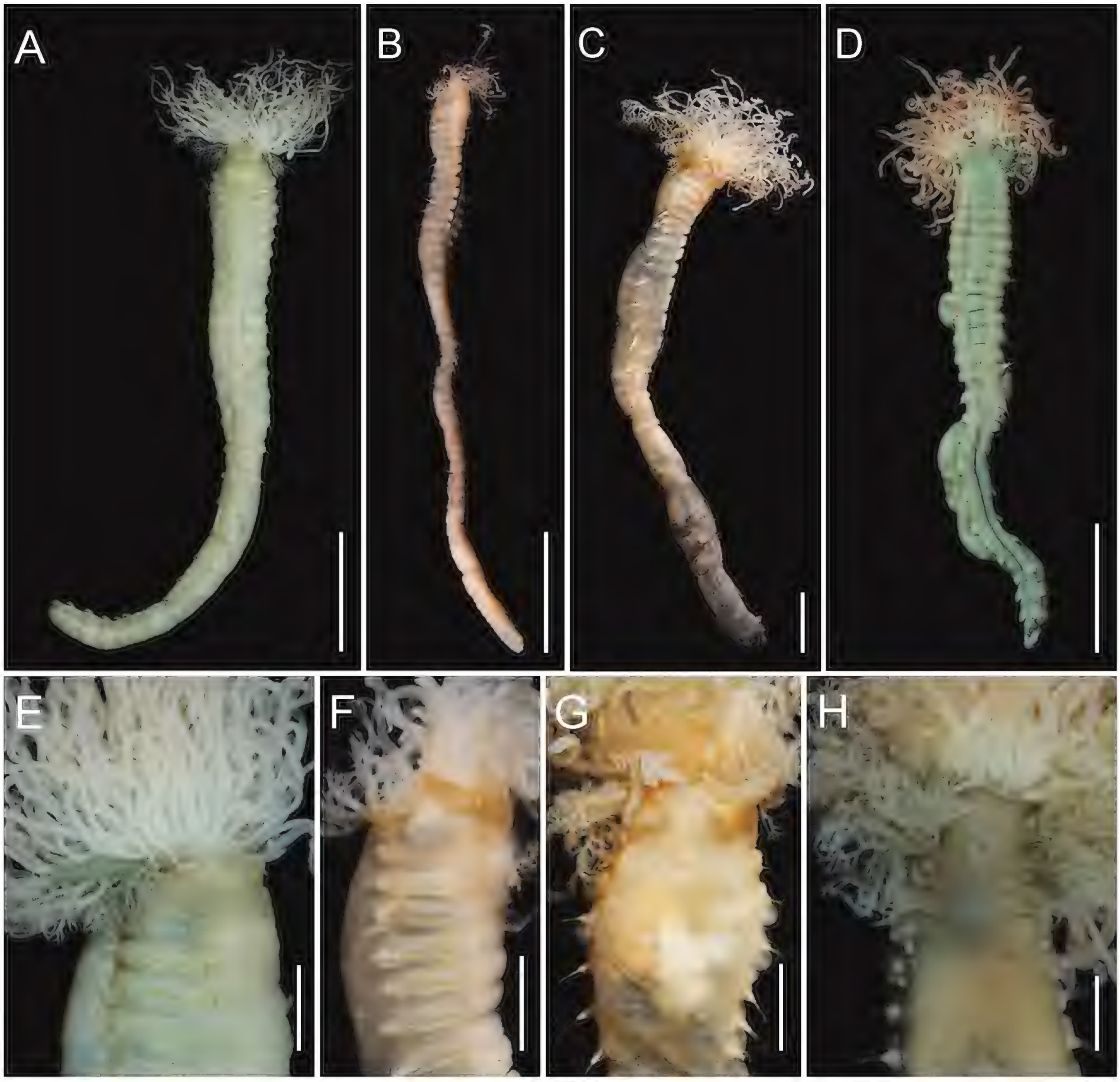

Figure 3. *Nicolea qingdaoensis* sp. nov. (**A, D**) ventral view. (**B, C**) lateral view. (**E, F**) Anterior, lateral view. (**G, H**) Anterior, dorsal view. **A, E.** QD_HZF_20250920 (MBM287950); **B, F.** QD_Terebellidae_sp2_002_250924 (MBM287948); **C, G.** QD_Terebellidae_sp2_003_250924 (MBM287948); **D, H.** QD_XZQ_Terebellidae_sp2_250917 (MBM287949). Scale bars: 5 mm (**A, B, D**); 2 mm (**C**); 1 mm (**E–H**).

Distribution. Known only from the intertidal areas of Qingdao, including new Zhanqiao Pier (36.05519°N, 120.34604°E, low tide zone), Hongshiya (36.08363°N, 120.09666°E, high tide zone) and Haizhifeng (36.06299°N, 120.38952°E, low tide zone).

Remarks. Only one species, *N. sinensis*, has previously been recorded from the Chinese coast. *Nicolea sinensis* was originally described from the subtidal of Che-Foo (Yantai, Shandong, China), and is characterized by four pairs of genital papillae in segments 6–9, glandular in females, and elongated in males (Fauvel 1932; Nishi et al. 2014). Later, specimens of *N. sinensis* were collected from Daheilan, Qingdao, which is approximately 195 km southwest of the type location (Fig. 4). Although these specimens were deposited in the MBMCAS (MBM199353), they were never formally reported. Given that the type specimen of *N. sinensis*, which was deposited in Paris, is not currently available, we re-examined these specimens from the MBMCAS collection and confirmed their identification as *N. sinensis* based on morphological characters provided by Fauvel (1932) (Fig. 4). The new species *N. qingdaoensis* sp. nov., can be distinguished from *N. sinensis* by having two pairs of genital papillae and there are four in *N. sinensis* (Fig. 4B, C) and a different dental formulae of uncini (MF:3–4: 2–3) in the new species and MF:2–3:1–2 in *N. sinensis*. In summary, *N. qingdaoensis* sp. nov., is characterized by 17 pairs of notopodia, ventral shields

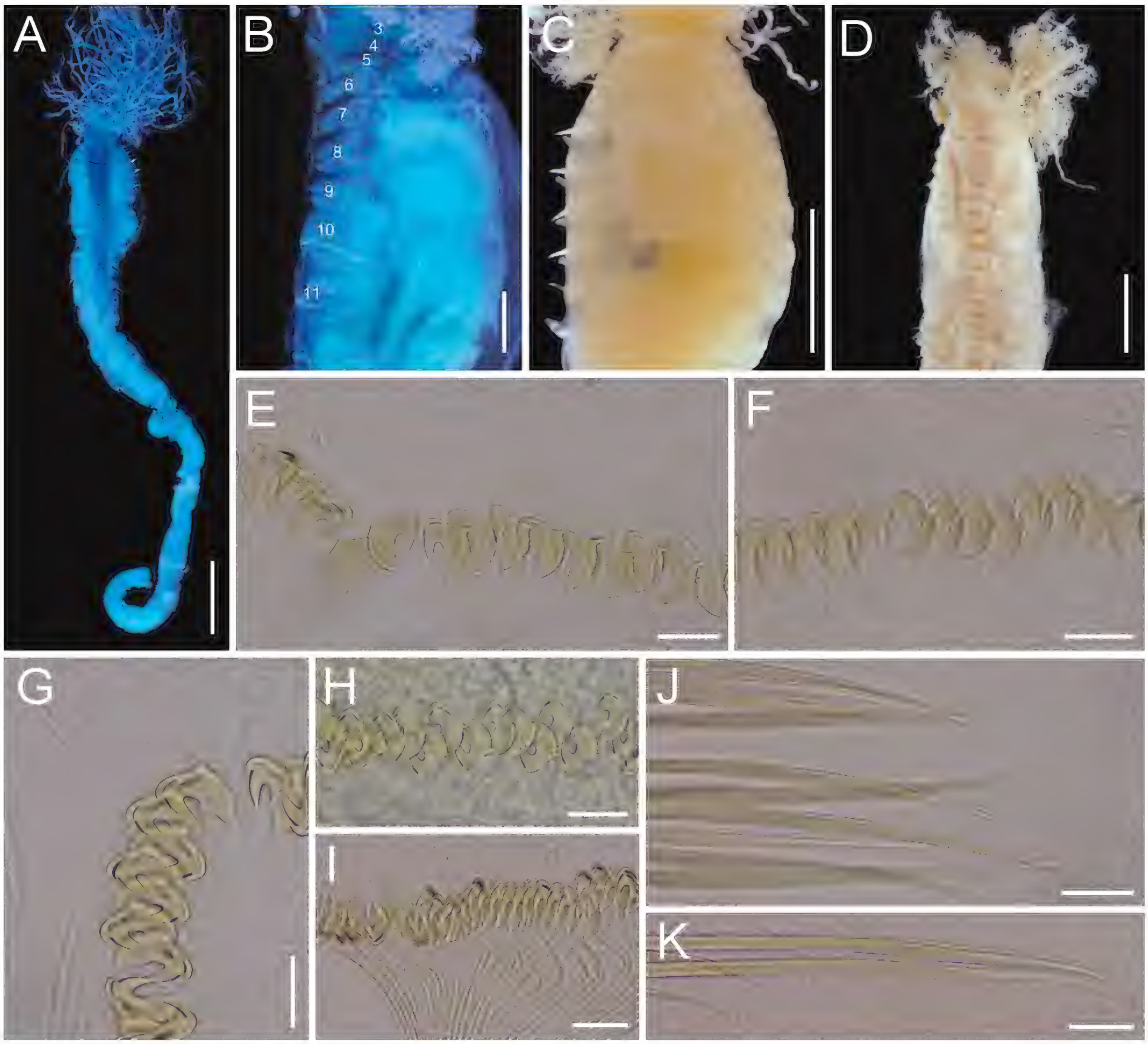

Figure 4. *Nicolea sinensis* Fauvel,1932. **A–C, E–K.** MBM199353 (Collected in Daheilan, Qingdao, Shandong, on Feb. 28, 1964); **D.** MBM190135 (Collection information is missing). **A.** Female, ventral view of whole specimen; **B.** Same specimen with **A.** Lateral view showing the genital papillae; **C.** Male, anterior, showing the genital papillae; **D.** Male, ventral view; **E.** Uncini, segment 6; **F.** Uncini, segment 10; **G.** Uncini, segment 11; **H.** Uncini, segment 18; **I.** Uncini, segment 27; **J, K.** Notochaetae, segment 18; **E, F.** From the specimen of **C**. Scale bars: 5 mm (**A**); 1 mm (**B**); 3 mm (**C, D**); 50 μm (**E–K**).

on segments 2 to 17, eyespots present, completely intercalated double rows of uncini starting from segment 11, and two pairs of genital papillae, spherical in females and digitiform in males.

Moreover, the type specimen of *N. sinensis* was collected from muddy dredged samples (Fauvel 1932), indicating a probable subtidal origin. The record of *N. sinensis* from the Tokyo Bay, Japan, was also from the subtidal mud (Nishi et al. 2014). While the new species *N. qingdaoensis* sp. nov., was collected from the intertidal rocky shore (although Hongshiya is mostly a muddy shore, the specimens from this site were collected from the high-tide area with rocks). Notable that the type specimen of *N. sinensis* has not been examined in either of the studies by Nishi et al. (2014) or this study. A definitive molecular and morphological comparison with material of *N. sinensis* is required. To achieve this, we plan to collect fresh specimens from its type locality, collect the molecular data and re-examine morphological characters. This will provide the basis for a systematic comparative analysis in a future integrative study.

The *N. qingdaoensis* sp. nov., is distinguished from its Western Pacific congeners by a suite of morphological traits, including body size, number of notopodia, number of segments with ventral shields, presence of eyespots, segment on which double rows of uncini begin, and uncinal morphology. In detail, there are 17 thoracic notopodia in *N. qingdaoensis* sp. nov., compared to 16 in *N. amnis*, 15 in *N. armilla*, and 14 in *N. murrayae*. Eyespots are present in *N. qingdaoensis* but absent in *N. angustiscutis*, *N. koehleri*, *N. longibranchia*, and *N. maxima*. The number of segments bearing ventral shields is 16 in *N. qingdaoensis* sp. nov.

(2 to 17), compared to 14 in *N claparedii* and *N. vaili* (2 to 15), and 19 in *N. willeyi* (2 to 20) (Table 1). *Nicolea qingdaoensis* can be distinguished from *N. gracilibranchis* by the extraordinarily large number and fineness of the tentacles, long and richly developed gills on a slender stem and branches, and extraordinarily wide uncinigerous pads in the latter species (Grube 1878).

Molecular comparison

The complete mitochondrial genomes of the four sequenced specimens are 15,810 bp (QD240920SP2_01), 15,780 bp (QD_Terebellidae_sp2_003_250924), 15,723 bp (QD_Terebellidae_sp2_002_250924), and 15,698 bp (QD_HZF_20250920) in length, comprising 13 PCGs, 23 tRNAs (including two trnM). All PCGs are encoded on the heavy strand (H-strand). All PCGs initiate with ATG and terminate with a complete stop codon TAA, except *nad1* (stop codon TAG), and *nad5* (stop codon TA, TAG, T) (Fig. 5, Suppl. material: table S3). The gene order of *N. qingdaoensis* sp. nov., is consistent with most of the other terebellids (Fig. 6). The gene order is relatively conserved in this family, except for two tRNAs. There are two *trnM* in most of the terebellid species except for *Thelepus plagiostoma* and a transposition of *trnS2* and *trnA* is present in this species.

Since the low sequence distance of *cox1* between *N. venustula* (Montagu, 1819) from the Mediterranean Sea and *Nicolea* sp. C from California, USA (0.5%), and between *N. zostericola* collected from Alaska, USA and Manitoba, Canada (2.5%), they were regarded as the same species, respectively. The pairwise genetic distance analysis across 12 *cox1* sequences of *Nicolea* showed that the interspecific genetic distances varied from 4.4% to 30.3%, and the intraspecific genetic distance of *N. qingdaoensis* sp. nov. (0.9% to 1.8%) (Table 2). The new species, *N. qingdaoensis* sp. nov., showed the smallest *cox1* divergence (11.4%) compared to *Nicolea* sp. C from Redondo Beach, USA (Table 2). For the *18S* gene, *N. qingdaoensis* sp. nov. showed a mean divergence of 18.25% (ranging from 17.3% to18.9%) against *N. zostericola* collected from Heligoland, Germany, and 22.75% (21.9% to 23.2%) against *N. uspiana* (Nogueira, 2003) collected from the Eastern Arabian Sea. Notably, the *18S* sequence attributed to *N. uspiana* is from specimens collected far from its type locality in the state of São Paulo, Brazil, casting doubt on the identification. The corresponding identities of *18S* among these specimens are 83.3% to 84.5% (*N. qingdaoensis* sp. nov., vs. *N. zostericola*) and 80.2% to 81.1% (*N. qingdaoensis* vs. *N. uspiana*), respectively (Table 3). The *18S* intraspecific divergence of *N. qingdaoensis* sp. nov., is 1.0% to 3.7%.

Terebella lapidaria OX424571.1 (15,973 bp)
Neoamphitrite affinis MZ326700.1 (15,823 bp)
Pista cristata EU239688.1 (15,894 bp)
Pista sp. PQ824720.1 (10,665 bp)
Lanice conchilega OZ221965.1 (15,771 bp)
Loimia medusa NC088773.1, OM809695.1 (16,023 bp)
Loimia arborea PP718025.1 (16,023 bp)
Thelepus plagiostoma MW557377.1, NC_060301.1 (15,628 bp)
Amaeana sp. PV263602.1 (16,002 bp)
Nicolea qingdaoensis sp. n. QD240920SP2_01 PX527395 (15,810 bp)
Nicolea qingdaoensis sp. n. QD_HZF_20250920 PX621371 (15,697 bp)
Nicolea qingdaoensis sp. n. QD_Terebellidae_sp2_002_250924 PX621372 (15,722 bp)
Nicolea qingdaoensis sp. n. QD_Terebellidae_sp2_003_250924 PX621373 (15,779 bp)
cox1 N cox2 D atp8 Y G cox3 Q nad6 cob W atp6 R H nad5 F E P T nad4l nad4 C M1 M0 rrnS V rrnL L1 A S2 L2 nad1 I K nad3 S1 nad2

Figure 5. Comparison of gene orders of Terebellidae mtgenomes. Conserved gene clusters in Annelida are indicated by different colors. Black dots: the putative control region is located. Black rectangles: missing tRNA in the corresponding species.

Table 2. Matrix of sequence divergence (*p*-distance, below the diagonal) and identity (above the diagonal) of *cox1* sequences among *Nicolea* species.

	1	2	3	4	5	6	7	8	9	10	11	12
1. *Nicolea qingdaoensis* sp. nov. (QD240920SP2_01)		98.5	98.3	98.4	84.7	85.4	89.3	76.6	86.4	85.2	89.4	85.1
2. *Nicolea qingdaoensis* sp. nov. (QD_HZF_20250920)	1.5		99.1	99.0	85.1	86.1	89.3	76.5	86.4	85.6	89.2	86.1
3. *Nicolea qingdaoensis* sp. nov. (QD_Terebellidae_sp2_002_250924)	1.8	0.9		99.0	84.7	85.7	89.5	76.3	85.9	85.6	89.4	85.8
4. *Nicolea qingdaoensis* sp. nov. (QD_Terebellidae_sp2_003_250924)	1.6	1.0	1.0		84.5	85.4	89.3	76.8	85.9	85.1	89.2	85.6
5. *Nicolea* sp. B	17.2	16.7	17.2	17.4		91.9	85.3	76.0	84.0	91.8	84.7	90.9
6. *Nicolea zostericola* (USA)	16.4	15.5	16	16.4	8.6		84.5	76.3	84.2	95.7	84.1	97.5
7. *Nicolea* sp. C	11.6	11.6	11.4	11.6	16.5	17.5		77.4	87.8	84.7	99.5	84.3
8. *Nicolea* sp. A	28.1	28.3	28.6	27.8	29.2	28.6	27.1		75.2	76.0	77.4	76.7
9. *Nicolea gracilibranchis*	15.2	15.2	15.9	15.9	18.2	18.0	13.5	30.3		83.5	87.9	83.5
10. *Nicolea* sp. D	16.7	16.2	16.2	16.9	8.7	4.4	17.4	29.0	18.9		84.5	94.7
11. *Nicolea venustula*	11.6	11.8	11.6	11.8	17.2	18.1	0.5	27.1	13.3	17.5		84.2
12. *Nicolea zostericola* (Canada)	16.8	15.5	15.9	16.1	9.8	2.5	17.8	27.9	19.0	5.5	18.0	

Table 3. Matrix of nucleotide sequence identity (above the diagonal) and divergence (below the diagonal) of *18S* from *Nicolea* species.

	1	2	3	4	5	6
1. *Nicolea qingdaoensis* sp. nov. (QD240920SP2_01)		97.8	97.2	99.0	83.4	80.4
2. *Nicolea qingdaoensis* sp. nov. (QD_HZF_20250920)	2.3		98.5	98.0	84.0	80.3
3. *Nicolea qingdaoensis* sp. nov. (QD_Terebellidae_sp2_002_250924)	2.8	1.6		96.4	84.5	81.1
4. *Nicolea qingdaoensis* sp. nov. (QD_Terebellidae_sp2_003_250924)	1.0	2.1	3.7		83.3	80.2
5. *Nicolea zostericola* (Germany)	18.8	18.0	17.3	18.9		84.8
6. *Nicolea uspiana*	22.9	23.0	21.9	23.2	17.1	

Phylogenetic analysis

The *cox1*-based phylogenetic analysis revealed that all seven species of *Nicolea* were placed in the clade Lanicini. Six of the seven *Nicolea* species formed a well-supported monophyletic clade (ML/BI: 100/1), which was sister to a clade containing *Pista* with a low support value (<50/<0.5) (Fig. 6, Suppl. material: fig. S2). Within the clade, *Nicolea qingdaoensis* sp. nov., was sister to a clade containing *N. venustula*, *Nicolea* sp. C, and *N. gracilibranchis*, with a good support (ML/BI: 97/1) (Fig. 6). The concatenated dataset of five genes (i.e. *cox1*, *16S*, 18*S*, *28S*, *H3*) comprised 6,363 bp, including 1,572 bp of *cox1*, 1,625 bp of *16S*, 2,116 bp of *18S*, 710 bp of *28S*, 340 bp of *H3*. Phylogenetic analyses based on the concatenated dataset were congruent with the monophyletic subfamilies Thelepodinae and Terebellinae (Fig. 7, Suppl. material: fig. S2). Within Terebellinae, four tribes, namely Polycirrini, Terebellini, Procleini and Lanicini, were recovered, consistent with that in the phylogeny by Stiller et al. (2020). The eight *Nicolea* species analyzed were placed in the Lanicini clade, among which, *N. uspiana* collected from the Eastern Arabian Sea was placed as sister to a main clade representing the genus *Lanice*, suggesting an incorrect identification. The remaining seven species formed a well-supported separate clade (ML/BI: 100/1; Fig. 7) and were placed as sister to the *Pista wui* with low support (<50/<0.5). The new species *N. qingdaoensis* sp. nov., was placed as sister to the clade of *N. venustula* collected from the Mediterranean and *Nicolea* sp. C from Redondo Beach, USA with good support (ML/BI:99/1) (Fig. 7, table S2). While *Nicolea* sp. A collected from Kaneohe Bay, USA was not grouped with most *Nicolea* species but was unexpectedly recovered as sister to *Terebella banksyi* Lavesque, Daffe, Londoño-Mesa & Hutchings, 2021 collected from France (Suppl. material: fig. S2).

Discussion

The new species collected in Qingdao in this study were assigned to *Nicolea* based on the diagnostic combination of the presence of two pairs of branchiae from segments 2 or 3, tabsence of anterior lateral lobes, 17 notopodia (segment 4 to 20), neuropodia beginning on segment 5, uncini short-handled throughout and the double rows of uncini from segment 11, notochaetae narrowly-winged, nephridial papillae on segment 3 and genital papillae on segment 6 and 7 (Hutchings et al. 2020). Nevertheless, *Nicolea* is one of the many genera in need of taxonomic revision. Many

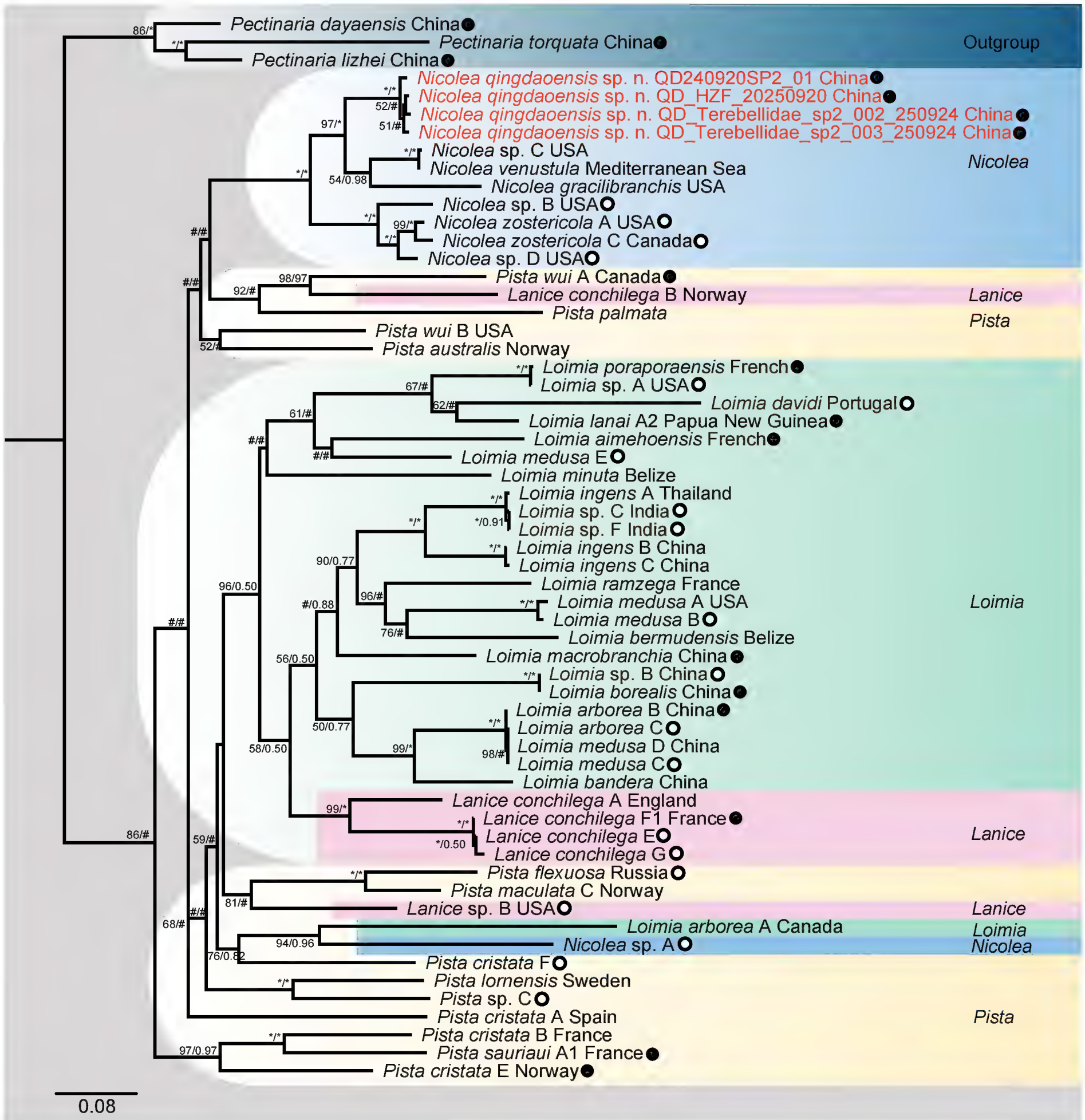

Figure 6. Phylogenetic tree based on *cox1* by ML/BI methods. The ML tree was displayed. Bootstrap support values of ML (left) and posterior probability of BI (right) are indicated above the nodes. Asterisks stand for maximum supports and pound keys stand for < 50 or < 0.5 for BI or unsupported values. The different colors represent the different genera of Lanicini and the outgroups of this evolutionary tree. Blank circle indicates that the straight-line distance between the collection site of the sequenced specimen and its type locality is unknown. The solid circle indicates that the direct distance of the collection site of the sequenced specimen and its type location is no longer than 500 km. The others without remarks are more than 500 km. The locality for some species is absent because the information was not recorded clearly or is missing in the original publication.

original descriptions are brief, with only a few illustrations and often lack diagnostic characters for identification, such as the presence or absence of lateral lobes, distribution and types of branchiae, the arrangement of uncini, and the appearance of nephridial and genital papillae. Furthermore, some species currently classified under *Nicolea* do not align with the generic diagnosis. For instance, *N. macrobranchia* (Schmarda, 1861) and *N. gracilicauda* (Kinberg, 1866) have three pairs of branchiae, whereas the genus *Nicolea* is characterized by having two pairs. Moreover, the reliability of some diagnostic characters is compromised by their variability. For example, a key character, the double rows of uncini starting from segment 11, begins from segment 10 in several species of *Nicolea*, such as *N. ceciliae* (Santos, Nogueira, Fukuda & Christoffersen, 2010), *N. murrayae*, and *N. vaili*.

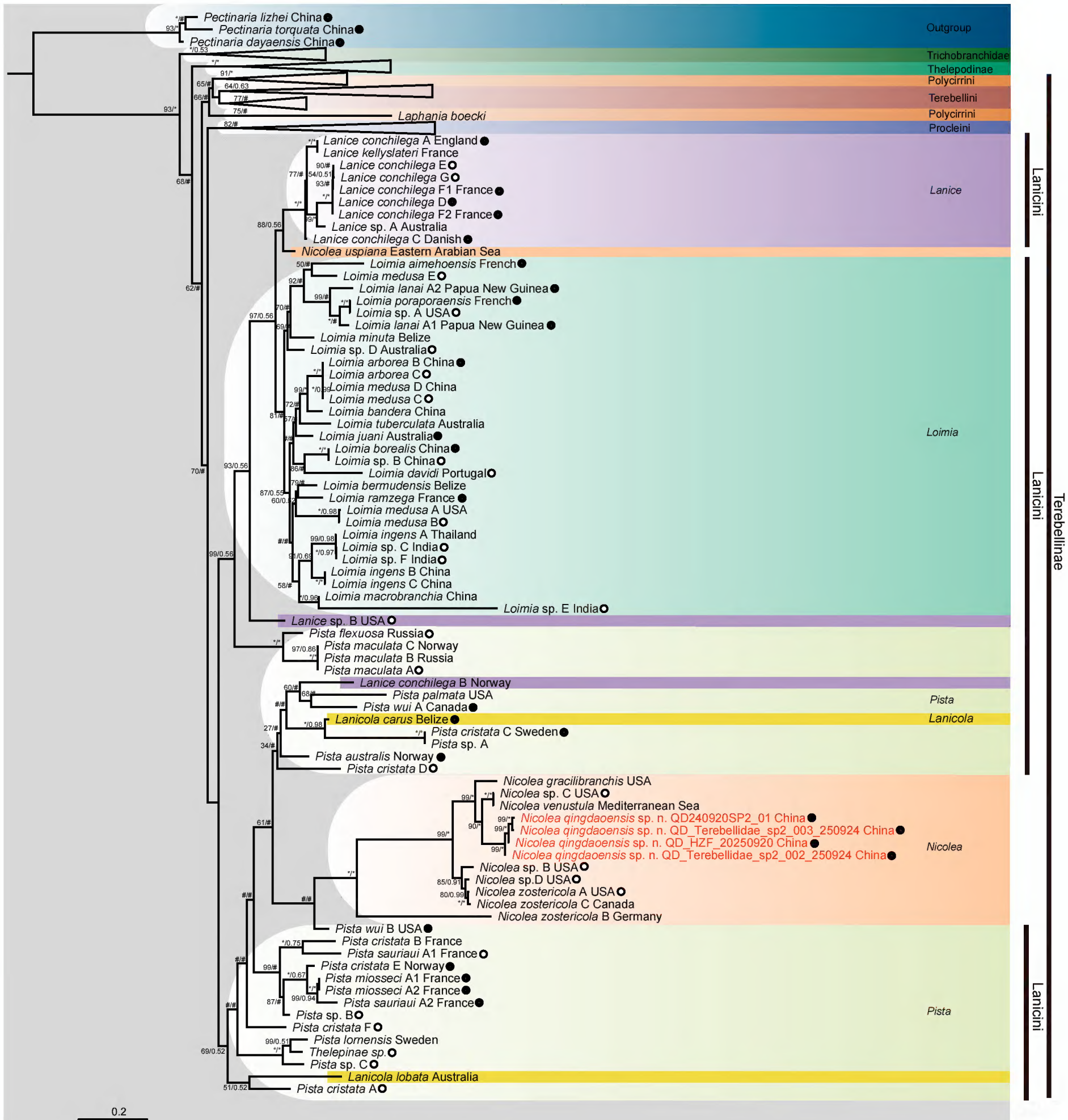

Figure 7. Phylogenetic tree based on concatenated five genes (*cox1*, *16S* rDNA, *18S* rDNA, *28S* rDNA and *H3*) by ML methods. Specimens of the red words are *Nicolea qingdaoensis* sp. nov. Terebellidae is divided into two subfamilies, Thelepodinae and Terebellinae. Terebellinae are divided into 4 tribes: Lanicini, Procleini, Terebellini, and Polycirrini, which are marked with text. Different genera in Lanicini are distinguished by different colors. Bootstrap support values of ML (left) and posterior probability of BI (right) are indicated above the nodes. Asterisks stand for maximum supports and pound keys stand for < 50 or < 0.5 for BI or unsupported values. The blank circle indicates that the straight-line distance between the collection site of the sequenced specimen and its type locality is unknown. The solid circle indicates that the direct distance of the collection site of the sequenced specimen and its type location is no longer than 500 km. The others without remarks are more than 500 km. The locality for some species is absent because the information was not recorded clearly or is missing in the original publication.

Besides, the diagnosis of *Nicolea* is challenged by the absence of a unique, defining character. Instead, the traditional diagnostic features of this genus comprise a combination of traits, each of which may be individually shared with other terebellid genera, thereby leading to indistinct taxonomic boundaries. For instance, the character suite historically used to define *Nicolea* – including two pairs of arborescent branchiae, notopodia distributed across 17 segments, and the presence of both nephridial and genital papillae on segments 3, 6, and 7 – is also found in some species of *Pista*, *Lanicola*, and *Neoleprea*. Similarly, the short and rounded handle of the intercalated uncini and

the absence of lateral lobes are features found not only in *Nicolea*, but also in *Terebella* or *Neoleprea* (Hutchings et al. 2020). This ambiguity extends to whether certain traits should be considered as generic or species characters. For example, 'two pairs of branchiae' is regarded as a fixed generic character of *Nicolea*, while in others, such as *Pista* and *Terebella*, the number of pairs of branchiae is a key feature for distinguishing species (Hutchings et al. 2021).

The significant *cox1* genetic distance between *N. qingdaoensis* sp. nov., and its available congeners – along with consistent morphological distinctions – provides clear evidence for supporting s a distinct species. Although there are no sequences of *N. sinensis* available for molecular comparison, the detailed morphological analysis supports that it is a new species. As in the description of *Loimia davidi* by Martin et al. (2022), specimens of dramatically different sizes can exhibit what appear to be interspecific morphological differences, yet belong to a single molecular species. In this context, molecular information confirms that specimens with different morphology, such as small papillae present on the branchiae and posterior region, are the same species. As highlighted by Hutchings et al. (2021), molecular data are most powerful for species delimitation only when sourced from voucher specimens collected at type localities, since sequences from misidentified specimens can introduce major taxonomic confusion. Sequences retrieved from GenBank derived from specimens collected far from their type locations may not correspond to the correct species and probably represent undescribed species (Lavesque et al. 2021). Herein, the specimen of *N. uspiana* (Nogueira, 2003) collected from the Eastern Arabian Sea is most likely misidentified due to its considerably distant to the type locality in Praia da Fazenda, Brazil, and its placement in a distinct phylogenetic clade separate from other *Nicolea*. Similarly, specimens identified as *N. zostericola* (Grube, 1860) from the USA, Canada and Germany, all geographically disjunct from the type locality, were placed in distinct phylogenetic clades, further indicating that their identifications require re-checking. Therefore, future studies should implement a more critical assessment of the reliability of sequences from public databases (e.g., GenBank) by verifying voucher specimens and their depositors, discarding problematic sequences, and prioritizing specimens collected from or near type localities to reconstruct a more reliable phylogeny.

Previous molecular phylogenies based on *cox1* have variably placed *Nicolea* either within a clade containing *Pista* and *Lanicola* (Lavesque et al. 2021), or as sister to a broader clade containing *Pista*, *Loimia*, *Amphitrite*, and *Neoamphitrite* (Choi et al. 2020). Our phylogenetic analyses support the placement of *Nicolea* within a clade comprising *Pista*, *Lanice* and *Lanicola*, even in single gene results (Suppl. material: fig. S3), consistent with Lavesque et al. (2021). It is worth noting that with a limited taxonomic representation for these genera, these phylogenetic inferences should be interpreted with caution. Moreover, the low support values for main clades underscore the need to incorporate molecular data from other morphologically similar taxa, to generate a robust and conclusive phylogeny. Additionally, future studies should prioritize more comprehensive data, such as mitochondrial genomes, over single genes like *cox1* or several rRNA genes.

Conclusion

In this study, we describe a new species, *Nicolea qingdaoensis* sp. nov., and provide its complete mitochondrial genome together with sequences of the nuclear markers *18S*, *28S*, and *H3*. The species can be distinguished from other members of the *Nicolea* by a combination of morphological characteristics, including 17 pairs of notopodia, ventral shields present from segment 2 to 17, the presence of eyespots, completely intercalated double rows of uncini beginning on segment 11, and two pairs of equal-length genital papillae on segments 6 and 7 that are spherical in females and digitiform in males. Phylogenetic analyses based on concatenated genes of *cox1*, *16S*, *18S*, *28S* and *H3* showed that species of *Nicolea* form a well-supported clade and belong to the tribe Lanicini. Given the absence of any comprehensive taxonomic study of terebellids from Chinese waters, the new species described herein represents only the tip of the iceberg.

Author contribution statement

HC prepared the figures and tables and drafted the manuscript. YZ collected the samples, photographed the living specimens and processed the sample preservation. YZ and YS designed the research. PH, YZ and YS edited the manuscript. All authors approved the submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material 1

Spplementary tables and figures

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Data type: xlsx

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